STUDIES ON FOOT-ROT AND LEAF-ROT OF *PIPER BETLE* L.—HOST RANGE AND ROLE OF CUTTINGS IN THE SPREAD OF THE DISEASE

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In an earlier paper (Mehrotra and Tiwari, 1967) the behaviour of the pathogen *Phytophthora parasitica** var. *piperina* re ponsible for foot-rot and leaf-rot of *Piper betle* Linn. has been described with respect to survival in the soil.

The present paper mainly deals with the comparisen of the pathogenic isolates from various sources (irrigation water, infected soil and collateral wild host), the mode in which the fungus attacks the host whether systemic or localized and finally the role of cuttings in the dissemination of disease.

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*(*a*) On the basis of morphological and physiological investigations, Thomas and Ramkrishnan (1948) have grouped *P. parasitica*, *P. palmivora*, *P. colocasiae* and *P. parasitica* var. *piperina* under *Phytophthora colocasiae* (Racib.) Thom. and Ram.

(*b*) Because of priority, Waterhouse (1963) proposes *P. parasitica* be changed to *P. nicolianae* var. *parasitica*.

OBSERVATIONS

In one of the experiments the fungus from different sources, *i.e.*, underground parts and aerial parts of 'Pan' (*Piper betle*), irrigation water and aerial parts of wild weed *Colocasia esculenta* (L.) Schott was isolated for comparison of their morphology and pathogenicity.

Bits of infected portions of leaves, roots and stems, etc., of *Piper betle* and *Colocasia esculenta* were surface-sterilized with 1% silver nitrate solution and pushed into potato dextrose agar and oatmeal agar plates. The fungus which appeared was isolated for further tests.

Phytophthora was isolated from irrigation water, by floating in it such baits as leaves, stem and root pieces of *Piper betle* together with hemp seeds.

The results were invariably positive. The isolates were grown in culture dishes. Their rate of growth, characters of the colony and sporangial measurements were recorded (Table I). Besides these, four

TABLE I

Colony characters, sporangial and zoospore characters of 7 *isolates of* Phytophthora

Isolate	Character of the colony	Size of the colony on oatmeal agar after 5 days incubation at 26° C. (cm)	Shape and size of the sporangia	Zoospore measurement
From aerial parts of Pan ..	Appressed with concentric zones	5·5	Pear-shaped or broadly ovate, sometimes elongate, 25–65 × 20–45 μ	9·6–12 μ in diameter
From underground parts of Pan	do.	5·2	Pear-shaped or broadly ovate, sometimes elongate, 25–70 × 20–50 μ	do.
From irrigation water ..	do.	5·2	Fear-shaped or broadly ovate, sometimes elongate, 25–60 × 20–50 μ	do.
From aerial parts of *Colocasia esculenta*	do.	5·2	Globose to ovoid, 25–50 × 26–40 μ	do.
P. parasitica var. *nicotianæ* (885)	do.	5·2	Globose to pear-shaped, 36·5–58 × 26·5–33·2 μ	do.
P. parasitica (884) ..	Velvety with faint zonations	6·5	Globose to ovoid, 30–50 × 25–30 μ	do.
P. parasitica (886) ..	do.	7·0	Globose to ovoid, 29·9–64·7 × 26·5–29·9 μ	do.

isolates of *Phytophthora*, *i.e.*, from aerial parts of *Piper betle*, underground parts of *Piper betle*, aerial parts of *Colocasia esculenta* and water of irrigation pond which were found directly associated with *Piper betle* at Sagar. 3 more isolates received from I.A.R.I., New Delhi, were also studied for comparison.

It will be seen from Table I that these isolates do not differ from one another in their morphology and cultural characteristics in any substantial way. The isolates from underground parts of 'Pan' (*Piper betle*) were inoculated on its leaves with successful results. Similarly, the isolates from the 'Pan' leaves were made to produce the foot-rot successfully indicating that leaf-rot and foot-rot organisms were one and the same. The isolate from *Colocasia esculenta* was tried on 19 hosts along with the isolate from 'Pan' for testing a broad spectrum of pathogenicity. The data are presented in Table II. It is notable that isolates of *Phytophthora* from *Piper betle* and *Colocasia* were wholly identical in their pathogenic behaviour while the rest of the three isolates which were received from I.A.R.I. behaved more or less similarly among themselves.

Another experiment was done in order to ascertain if the infection was systemic or localized.

Healthy plants of *Piper betle* were inoculated in pots. The inoculum was mixed with the soil and favourable conditions of moisture and temperature were provided for development of foot-rot. Symptoms of foot-rot appeared in about 20–25 days and the plants died after a few days. However, no rotting spots were visible on the aerial parts. Small pieces of plant parts from different heights were plated to detect the pathogen but only with negative results. The tissues of *Piper betle* parts, stem and leaf were also examined microscopically by cutting hand and microtome sections but no fungus could be detected. On the other hand, if the aerial parts of the plant were inoculated the infection travelled downwards reaching the soil level and caused foot-rot, the fungus could not travel upwards since the top above the necrotic spot got severed off due to the rot. This experiment was done with optimal environmental conditions of temperature and moisture, *i.e.*, 18 to 22° C, with profuse watering.

In the third experiment the role of cuttings in the spread of disease was studied.

Leaf-rot.—Eight small young plants (established cuttings) were taken with roots and were washed well. These plants were steeped completely in mycelial and zoospore suspension and then kept erect in small flasks in which some water was put to prevent wilting. These flasks were put under room conditions (20 to 25° C.) for variable lengths of time (treatments). The time allotted to different plants was 5, 10, 20, 40, 80, 160, 320 and 640 minutes. After these treatments the plants were put in the pots with natural soil and transferred to con-

TABLE II

Results of pathogenicity experiments

Host	*P. parasitica* var. *piperina*	*P. parasitica* var. *nicotianæ* (885)	*P. colocasiæ*	*P. parasitica* (884)	*P. parasitica* (886)
Piper betle ..	+++	+	+++	+	+
Piper longum ..	++	+	++	+	+
Nicotiana tabacum ..	+++	+++	+++	+++	+++
Lycopersicon esculentum ..	++	++	++	++	++
Jatropha sp. ..	+++	+++	+++	+++	+++
Carica papaya ..	–	–	–	–	–
Martynia annua ..	++	++	++	++	++
Xanthium strumarium ..	–	–	–	–	–
Solanum tuberosum ..	++	++	++	++	++
Capsicum annuum ..	–	–	–	–	–
Colocasia esculenta ..	+++	++	+++	++	++
Sauromatum guttatum ..	–	–	–	–	–
Ricinus communis ..	+++	+++	+++	+++	+++
Citrus medica var. *limonum* ..	+++	+++	+++	+++	+++
Psidium guajava ..	+	+	+	+	+
Basella rubra ..	++	++	++	++	++
Pepromia pellucida ..	+	+	+	+	+
Amaranthus gangeticus ..	–	–	–	–	–
Dioscorea alata ..	–	–	–	–	–

– = Immune, Inoculation was not successful, *i.e.*, the plant remained healthy; + = Moderately susceptible, spots confined to inoculated region; ++ = Susceptible, spots were not very extensive but travel to some distance; +++ = Highly susceptible, characteristic spots developed and rot extended almost to the whole leaf.

genial conditions of temperature and moisture in the glass-house to develop the disease.

It was observed that leaf-rot appeared only in those plants which were transferred to glass-house conditions within 40 minutes, *i.e.*, those, which were kept out for 5, 10, 20 and 40 minutes, developed the disease. Those plants which remained out in the room condition for more than 40 minutes failed to develop any leaf-rot disease because of the drying effect which killed the surface inoculum. This shows that even if cuttings of plants are surface-contaminated they would not develop any leaf-rot unless they are transferred to optimum conditions of disease development soon, *i.e.*, before the inoculum get dried up.

Foot-rot.—The plants as treated above (A) were also tried for foot-rot by placing them in soil in glass-house conditions. There was no question of any surface drying since the roots were kept dipped in water for requisite intervals of time.

Observations revealed that foot-rot developed in all cases excepting first two treatments, *i.e.*, 0 and 5 minutes. This could be explained by the fact that the surface inoculum of the fungus becomes ineffective in soil due to the interactions with the soil saprophytes in case of first two treatments. In the later treatments where plants were kept for longer time in water the disease developed because in these cases the pathogen had enough time to penetrate in the tissues which gave it a protection against soil micro-organisms.

Study of pathological anatomy.—The diseased stem and root pieces were examined by cutting hand and microtome sections in various planes. When tissues of an advanced stage of rotting were examined they were found to be consisting of a softened dark brown mass in which majority of the cell walls and cell contents were disorganised. When tissues adjoining to these rotted areas were examined by sectioning, partially disorganized cell walls were seen which were also filled with a brownish gummy substance. Mycelium, both intercellular as well as intracellular, was found in the parenchymatous cells of the cortex in this region. Rarely mycelium was also seen in the lumen of the vessels. In examining the zone even beyond this more or less healthy cells could be seen in which some (much less) brownish substance was present, but no mycelium was discernible. These cells appear to be affected in advance of the oncoming mycelium of the pathogen probably due to the secretions of some toxins.

The histological observations showed that the mycelium of the pathogen is not found in distant zones away from the rotting places. The pathogen caused localised rots and was not present in the healthy tissues in general in a systemic way. This shows that the pathogen is not an advanced parasite and does not establish an equilibrium with the host.

Discussion and Summary

The diseases of foot-rot and leaf-rot of *Piper betle* have been under study in this laboratory for the last five or six years. In an earlier

paper the survival and certain other aspects of the disease were dealt with. In this paper experiments have been described to determine whether the fungus from collateral hosts like *Colocasia esculenta* was same morphologically and in pathogenic behaviour or not. It was noted that a wild *Colocasia* sp. grows nearabout the *Piper betle* orchards and is heavily infected with *Phytophthora*. It is suspected that this species of *Colocasia* plays the role of collateral host for the fungus found on *Piper betle*. In this study it was found that in a broad spectrum of pathogenicity in which the isolates were tried on 19 different hosts, the two fungi were identical in behaviour. Since they did not differ substantially in morphology also, it was concluded that the two are wholly identical. Side by side a few isolates of *P. parasitica* received from I.A.R.I. were also tried for comparison. These appeared to be slightly different from the main pathogen under study (Table II).

In another experiment the nature of pathogenesis was studied for finding if the fungus was systemic in its behaviour or causes only localised lesions. It was found that the fungus is not able to proceed upwards from a lesioned spot because the vine gets severed off and falls down. However, the fungus is able to descend down from the affected spots. The portions of the vine which fall down during the rot disease of the aerial parts then become a source for soil infection and cause the foot-rot. It was normally noted that in the orchards the first to appear was generally leaf-rot rather than foot-rot. It appears that the infection in the aerial parts comes most probably from outside, the irrigation water or otherwise.

In another experiment the role of cuttings in the initiation of the disease was examined, the results suggested that there is hardly any possibility of the disease being carried on the surface of the aerial parts excepting in evident lesions which can be detected easily. This finding is somewhat different from that of Asthana (1947) who believed that the disease is carried both externally as well as internally in the cuttings.

A preliminary study of the morbid anatomy of the affected parts has also been made, it revealed that this species of *Phytophthora* develops both intercellularly as well as intracellularly and kills the tissues in advance suggesting the production of some toxic substances. No haustoria could be seen. The pathogen is rather of a primitive type and not much adapted to its host.

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